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EXPERIMENTAL VITAMIN E DEFICIENCY

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EXPERIMENTAL VITAMIN E DEFICIENCY

More than twenty years have passed since the discovery of vitamin E yet, as Mason (1) remarks, it still remains "the mysterious intruder" in the vitamin family. To the chemist, vitamin E and its homologues are well known organic compounds which can readily be isolated from a wide variety of natural sources or synthesized in the laboratory. The biochemist, physiologist, nutritionist and clinician, however, are still confronted with a series of confusing questions concerning physiological responses to deficiencies of vitamin E or to its therapeutic administration.

It is known that vitamin E is involved in the physiology of the reproductive system of both male and female, as well as in the physiology of the muscular and vascular systems. It is thought to have a possible relation to certain endocrine dysfunctions and also to the development of characteristic lesions of the nervous system. Because results of animal experiments and of clinical studies have been so confusing, and frequently contradictory, many erroneous impressions have unfortunately arisen with respect to the biologic importance of this vitamin and only a few positive indications have been forthcoming concerning its true significance in the living organism. The fact that vitamin E seems to have a multiplicity of apparently unrelated functions has served to further complicate the picture. This Review will attempt to clarify the biological role of vitamin E in experimental animals in the light of present knowledge and experimental evidence.

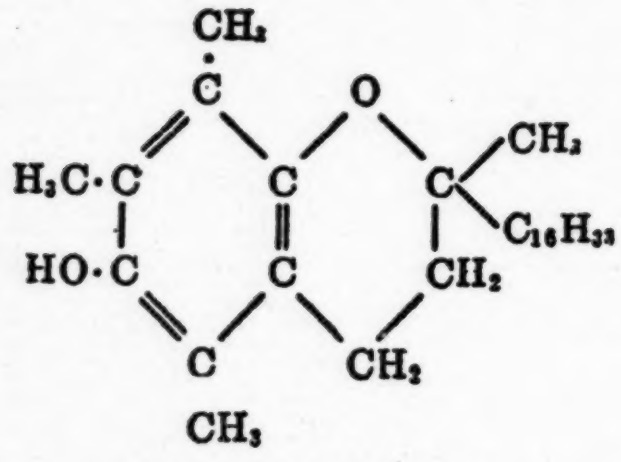
Discovery of Vitamin E:

In 1922, during studies on fertility in the rat, Evans and Bishop (2) observed that female rats reared on a purified diet containing all the vitamins known at the time failed to reproduce although they were apparently normal in all other respects. Mating and conception occurred normally in these animals, but pregnancy invariably terminated in early death and resorption of the fetus. At about the same time independent investigators in other laboratories confirmed these observations (3, 4, 5). The addition of lettuce, whole wheat or dry cereals to the diet restored the ability of the females to carry their pregnancies successfully to term with the birth of normal, live offspring. Evans and Bishop therefore suggested the existence of a new unidentified dietary factor essential to normal reproduction. This factor was called vitamin E by Sure (5). Later rat studies by Evans and Bishop revealed that lack of vitamin E not only resulted in fetal deaths in the pregnant female, but in a true and permanent sterility in the male.

Chemistry:

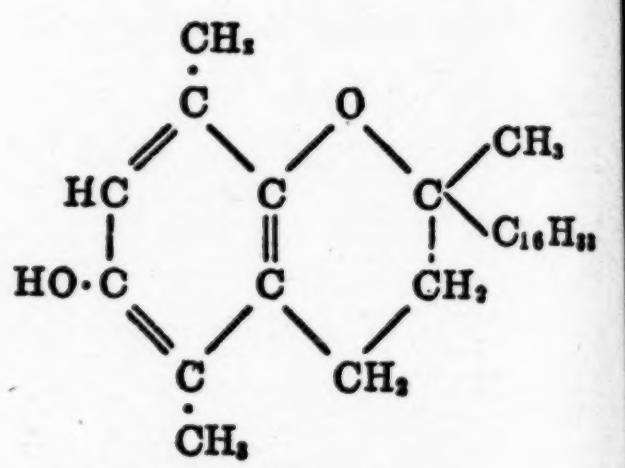
The earliest studies of the chemical nature of vitamin E were made in 1927 by Evans and Burr (6) who found wheat germ oil to be a potent source of this factor. About nine years later two alcohols were isolated in crystalline form from the wheat germ oil concentrates and found to possess vitamin E activity (7). These were identified as alpha- and beta-tocopherol. Since then, a third alcohol, gamma-tocopherol, has been added to the list of naturally occurring vitamin E active compounds. Elucidation of the structural formulas of the tocopherols (fig. 1) and their synthesis followed after a relatively brief period of intensive study by a number of organic chemists both in the United States and abroad (8).

(I)



alpha-Tocopherol

(II)



beta-Tocopherol

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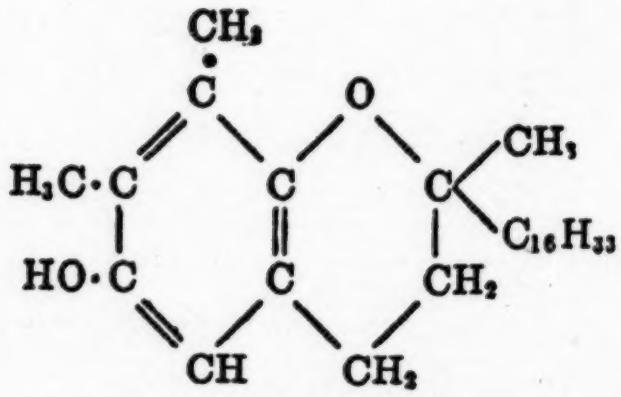

gamma-Tocopherol

Fig. 1

Of the three tocopherols, the alpha compound possesses the greatest biological activity; the beta and gamma forms have lesser potencies respectively, approximately ½ and 1/12 that of alpha-tocopherol (9). The natural tocopherols occur in plant materials and represent members of a large group of compounds known as "inhibitols," or antioxidants which are responsible for the stability of vegetable fats toward oxidative deterioration (10). The tocopherols are reported to be very effective as stabilizers of animal fats and of unsaturated fatty acids *in vitro* (1). It is doubtful, however, that their primary role in the animal body is related to their antioxidant properties, because the tocopherols themselves are very susceptible to oxidation

unless protected by other antioxidants. The interesting fact that the antioxidant activity of the three tocopherols is inversely proportional to their biological activity (11) would seem to be a further indication that the activity is not entirely related to this property.

(Homologues): Not only the tocopherols, but a number of other organic compounds – for example, coumarins, phenols, quinones – entirely unrelated in their molecular structures to the tocopherols and to each other, show marked vitamin E activity (12). Of especial interest in the chemistry of these vitamin E homologues is the lack of structural specificity in the various active compounds. On the other hand, a relatively slight modification in the structure of alpha-tocopherol itself may markedly diminish or destroy its biological activity.

Physiological Functions:

As Mason (1) has pointed out the most outstanding characteristic of experimental vitamin E deficiency, which has no parallel in any other vitamin deficiency state, is the multiplicity of structural and functional alterations exhibited. Added to this is the unusual fact that closely related species (as, for example, the rat and mouse) will exhibit in some cases, entirely different manifestations of vitamin E deficiency while other, quite unrelated species will show corresponding responses with respect to certain other vitamin E deficiency manifestations. Furthermore, in many deficiency states of other vitamins an understanding of the morphological changes occurring in cells and tissues constitutes a valuable guide in the approach to the problem of vitamin function. In the case of vitamin E deficiency, however, the situation is more complex. The pattern of change is not limited to a specific type of cell or tissue and therapy is not followed by an orderly process of structural repair.

Reproductive System: There seems to be more definite knowledge of experimental vitamin E deficiency as it is manifested in disorders of the reproductive system than exists with respect to any other phase of its biological activity. Most of the studies have been confined to the rat through which the existence of the vitamin was first discovered. Lack of vitamin E affects both male and female of the species, though in totally different ways. In the female there is no direct effect or injury to tissues of the reproductive system, but instead a definite interference with the development of the embryo. Thus, in female rats deficient in vitamin E, oestrus, ovulation, conception and implantation of fertilized ova occur normally. Degenerative changes occur, *not in the deficient female, but in the developing embryo,* which eventually succumbs and is resorbed (6). Vitamin E deficiency in the female rat, therefore, does not constitute absolute sterility, but

rather the inability to carry the fetus to term and thereby produce living young. Restoration of vitamin E to the diet before the end of the first half of pregnancy or prior to and during subsequent pregnancies enables the female to successfully bear offspring.

The cause of fetal death and resorption has been attributed to a suppressed development of the blood-forming tissues in the embryo (6, 13). Recent studies, however, based on examination of 16 day-old fetuses, have revealed no serious depression of such blood-forming functions. Rather, they have indicated that abnormalities of the vascular system – stagnation of the blood, distention and thrombosis of the veins – which lead to general ischemia and sometimes to actual hemorrhage, constitute the real causes of death (14, 15). These blood changes can be prevented by the administration of vitamin E, but do not respond to therapy with either vitamin C or vitamin K.

In the male, vitamin E causes direct and irreversible damage to the tissues of the gonads, thereby interfering with the production of spermatozoa and resulting in a true and permanent sterility (16). These changes occur at the onset of sexual maturity, the animal apparently suffering no ill effects during the adolescent period. Vitamin E deficiency in the male rat may be prevented by administration of the vitamin at weaning or by sufficient transfer and storage of the vitamin through mother's milk prior to weaning (17, 18). Prolonged therapy with vitamin E may result in some restoration of the injured seminiferous epithelium in deficient rats, but failure to restore fertility or to establish the presence of mature, functional spermatozoa has been consistently reported.

In the mouse, vitamin E deficiency likewise results in fetal death and resorption in the pregnant female, but strangely enough is without effect upon the male (19, 20). Male chicks (21) and guinea pigs (22) have also been reported to suffer testicular injury as a result of vitamin E deficiency.

Muscular System: The first indication that the effects of vitamin E deficiency are not restricted solely to the reproductive system came about in 1928 through some of the later studies of Evans and Burr (23). At that time, they conducted an experiment in which mother rats low in vitamin E were permitted to attempt the suckling and rearing of their young. These animals had been given barely more vitamin E than was necessary to insure the birth of living young, and were capable of successful lactation. The young grew normally and apparently throve. Suddenly, however, Evans (24) reports "toward the end of the lactation period a calamity intervened for we were surprised to find that the majority of these well-nourished young developed a mysterious malady, characterized mainly by muscular

paralyses. Half of the affected sucklings died from the malady – often so suddenly that there were no signs of wasting from malnutrition." The cause of this unexpected disorder was soon traced to a lack of sufficient vitamin E in the mother's milk. This was confirmed by the fact that young born of normal mothers plentifully supplied with vitamin E soon developed the disease, in mild form, if transferred to E-deficient females for suckling. Furthermore, administration of vitamin E (as wheat germ oil concentrates) to the nursing females or directly to the young effectively prevented the disease. These experiments established a hitherto unrecognized need for vitamin E in the economy of the developing young.

While the investigations of Evans and Burr continued, primarily along lines concerned with the chemistry of vitamin E, other workers were engaged in a study of the vitamin as it affected species other than the rat. In the course of these studies, the discovery was made by Goettsch and Pappenheimer (25) that rabbits and guinea pigs reared on a vitamin E deficient diet developed a specific deficiency disease characterized by dystrophy of the voluntary muscles. The disease was named by them "nutritional muscular dystrophy." Almost a decade later, Olcott (26), following up the studies of Evans and Burr on paralysis of suckling rats, discovered gross degenerative lesions of the muscles of these animals exactly similar to those occurring in guinea pigs and rabbits as described by Goettsch and Pappenheimer. This remarkable finding brought to light the fact that nutritional muscular dystrophy resulting from vitamin E deficiency can occur in other mammals, most significantly in the rat in which the paralysis was first noted. Since that time, other investigators have produced the disorder in a wide variety of animals (sheep, goats, mice, ducks, geese, hamsters, dogs) thereby extending the biological need for vitamin E to many species.

The interesting fact that the inclusion of cod-liver oil in the diet of certain herbivorous animals may frequently lead to the development of muscular dystrophy was a matter of controversy for some years. Originally, this peculiar effect of cod-liver oil was thought to be the result of a direct toxic effect of the oil (27), but it is now believed, as a result of extensive studies, to be due to the presence of unsaturated fatty acids in the oil which apparently promote the oxidative destruction of vitamin E either in the diet or in the digestive tract (28, 29).

Vascular System: In the case of chicks it has been found that vitamin E deficiency may result in profound changes in the vascular system leading to characteristic lesions of the brain, hemorrhage and eventually death. Among the earliest observations of the effect of

vitamin E on the vascular system were those of Adamstone (30) in 1931 on the embryonic development of chicks from hens fed on vitamin E deficient ration. He found that unless the chick egg contains an adequate amount of vitamin E at the time of laying, embryonic death occurs about the fourth day of the incubation. This was attributed to disintegration of certain blood vessels, hemorrhage, and the formation in one of the germ layers of embryonic tissue of a dense lethal ring which chokes off circulation.

About eight years later, two other serious disorders in vitamin E-deficient chicks were recognized and have since been related to disturbances in the vascular system. One of these, described as an "exudative diathesis" was first reported by Dam and Glavind (31, 32) in Copenhagen; the other, termed "nutritional encephalomacia," was originally described by Goettsch and Pappenheimer (33, 34) in this country. Both of these diseases occur in young chicks reared on purified vitamin E deficient diets. The disorder described by Dam and Glavind is characterized by an accumulation of a watery, plasma-like fluid under the skin, and in the muscles, and fatty and connective tissues. In nutritional encephalomacia, injury is confined chiefly to the brain. The symptoms include sudden ataxia (paralysis), retraction of the head and prostration associated with brain hemorrhages, thrombosis and degenerative softening of certain brain tissues. These, as well as numerous other histological lesions, have all been traced to circulatory disturbances as have the symptoms characteristic of exudative diathesis. In this connection it is noteworthy that the cause of fetal death and resorption *in utero* in E deficient rats has likewise been attributed by Mason (14, 15) to abnormalities of the circulatory system (see page 4). The disorders in the duck have been found to respond to treatment with alpha-tocopherol. Again, as in the case of dystrophic muscular disorders in other species, the presence of unsaturated fatty acids in the diet has been found to precipitate the characteristic disturbances in the chick (1). Dam and co-workers (35, 36) have shown further that a number of other dietary modifications, exclusive of vitamin E content, exert a marked effect on the rate of onset and general incidence of the symptoms in the chick. The influence of these dietary factors has served to explain in part the development of different reactions to vitamin E deficiency as reported by different groups of investigators using varying experimental rations and may also offer some insight into the mode of action of vitamin E.

Nervous and Endocrine Systems: Although vitamin E had been thought from the very earliest studies on muscular dystrophy to be associated with lesions of the nervous system, particularly of the spinal

cord, evidence to date has failed to successfully substantiate these views. Lipschutz (37), in 1936, was the first to report degenerative changes in the central nervous system of rats suffering the vitamin E deficiency paralysis originally noted by Evans and Burr. Olcott (26) who later correctly attributed the paralysis to degeneration of the voluntary muscles, failed to find any lesions in the cerebrospinal system. Since that time there have been many reports pointing to involvement of various parts of the nervous system, but these have been refuted by other equally numerous studies. Pappenheimer (38) attributes this lack of agreement to possible subtle differences in diet, technical procedure or even in interpretation. Both he and Mason (1) agree that in all probability there is no foundation for the supposition that the nervous system is involved in vitamin E deficiency. Mason states that although certain findings have pointed toward envolvement of the nervous system, negative findings have been reported in many other instances and "a searching re-examination by Wolf and Pappenheimer (39) has brought forth quite convincing evidence that the lesions previously described are due to some factor in the experimental procedure other than vitamin E."

Bcause of the nature of the reproductive disorders encountered in both male and female animals on vitamin E deficient diets, a great deal of speculation has arisen with respect to a possible relation between vitamin E and the various endocrine organs and their respective hormone secretions. Of particular interest in this connection have been the studies attempting to correlate the sterility of vitamin E deficiency with dysfunction of the anterior pituitary or, possibly, the thyroid glands. The preponderance of experimental evidence, however, seems to indicate no direct relation between the vitamin and the hormone system, the conclusion being that endocrine disturbances are more likely the result of, rather than the cause of reproductive disturbances associated with vitamin E deficiency. The vast literature dealing with studies along these lines has been outlined in several reviews (1, 40, 41).

Conclusion:

From the experimental evidence thus far available, a great deal of thought-provoking information has been gleaned concerning the importance of vitamin E in the economy of the animal body. Although its precise physiological role or roles are not yet entirely crystallized in scientific records, several outstanding characteristics peculiar to vitamin E have become recognized and give promise of becoming valuable clues in the further understanding of its action. These properties include the unique relationship of vitamin E to

apparently widely varying systems in the animal body – reproductive, muscular, vascular, and possibly others – a relationship not exhibited to such extent by any of the other vitamins. The variations in vitamin E behavior with sex and species, while confusing at first, may also contribute to an eventual explanation of its mode of action. A number of ingenious theories have already been forthcoming concerning the possible mechanisms by which the vitamin may act. Many of these, although still to be confirmed, have succeeded in tying together the apparently unrelated manifestations of vitamin E behavior and give promise of providing a clearer picture of these phenomena in the near future. These theories, as well as the practical implications of vitamin E in clinical medicine will be discussed in a future issue of this Review.

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NEWS DIGEST

ARMY DIET IN TROPICS—A study of the Army dietary was made in a forward area on an island in the Southwest Pacific. The study was prompted by an increasing number of soldiers who complained of loss of appetite, weight and energy, and an increase in irritability and sleeplessness. A quantitative study of the diet as provided and as consumed by a small group of soldiers revealed primary dietary deficiencies in calcium, thiamine, riboflavin, and ascorbic acid. Studies by other investigators suggest that certain additional conditioning factors may render more acute the effects of this primary lack. The secondary or conditioning factors of importance in the studies were the influence of anxiety states, the influence of tropical heat and humidity in reducing the desire for food, the effect of palatability in reducing the intake of foods, and the increased bodily requirements incident to an unusually high carbohydrate diet. These findings suggest that the dietary as provided to troops in the tropics be fortified with respect to the deficient elements. Such a program is said to be now under way whereby increased amounts of calcium and vitamins are being incorporated in flour and yeast. The supplemental use of polyvitamin preparations in that case would not be advised except when circumstances indicate a failure to obtain sufficient quantities of those substances in the food eaten.—*Bull. U. S. Army Med. Dept. 82,* 43, November, 1944.

CABBAGE—Cabbage commonly contains approximately a thousand times as much vitamin C as vitamins B_1 and B_2 according to recent studies. Plant scientists found that the six "wrapper" leaves invariably produced the greatest quantity of each of the vitamins. In May, for example, there was 1.5 times as much vitamin B_1, about 2.0 times as much vitamin C, and 3.7 times as much vitamin B_2 in the wrappers as in the outside leaves of the solid head, which in turn had a greater vitamin content than the innermost leaves. However, the relative positions shifted with the seasons. As the weather becomes cooler there is a tendency for vitamins B_1, B_2, and C to be higher in the younger, deeply protected leaves. The distribution of the three was more uniform throughout the head in the fall than in the spring, and the average vitamin content was greater in November than in May. It was pointed out that cabbage as a vitamin source has often suffered in comparison with other leafy vegetables, but that the wrapper leaves of cabbage, which correspond to the large leaves of turnip and kale, produce just as much or even more of all three vitamins.—*Jour. Am. Diet. Assoc. 21,* 120 (1945).

CONSUMPTION OF FOOD BY WACS—At the time of the organization of the Women's Army Corps, it was expected that women would consume less food than men, not only because their assignments were normally lighter, but also because civilian women's eating habits differ from those of men. Therefore, early in 1944, the Food Service Branch initiated a controlled food consumption survey to determine what percentage of food quantities allotted for men were actually consumed by the WACs. The survey results, based on consumption and desirability, are of interest not only to WAC installations, but to civilian feeding establishments. The findings show how WACs tend to "stylize" the Army mess. WACs do not consume as much coffee as do servicemen. Canned and fresh fruit juices are very popular. Women prefer less cereal than men, the amount varying according to the season. The issue of bread in a WAC mess hall is 20 per

cent less than for men. Women consume 50 per cent less macaroni and rice and breakfast sausage, and prefer smaller portions of most meats.—*J. Am. Diet. Assoc. 21,* 83 (1945).

B COMPLEX IN MILK – Data concerning the quantitative presence of the lesser B vitamins in milk have recently been made available by newly developed microbiological assay methods for these vitamins. The average niacin, pantothenic acid, choline and biotin content of fresh milk was found to be 0.91 mg., 3.1 mg., 149 mg. and 47.1 micrograms per liter, respectively. No significant losses of these four vitamins were found to occur in the processing of irradiated evaporated, dry skim or dry whole milk. Possibility of small and probably insignificant processing losses can not be definitely excluded because the raw milk sources of the different types of milk tested were not identical, because of the variability of the data, and because of the limitations in accuracy of the microbiological methods used.—*J. Nutr. 29,* 137 (1945).